

Title: COMPOSITIONS INVOLVING M-RIP, AND RELATED  
METHODS FOR SCREENING FOR ANTI-HYPERTENSIVE  
AGENTS, AND USES THEREOF

Applicant(s): Michael E. Mendelsohn et al.

Filing Date: 11/17/03

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1A

Human: 1	MSAAKENPCRKFQANIFNKSQCNCFKPREPHLLNDEDLTQAKPIYGGWLLLAPDGTDFD	60
Mouse: 1	MSAAKENPCRKFQANIFNKSQCNCFKPRE HLLNDEDLTQAKPIYGGWLLLAPDGTDFD	60
Human: 61	NPVHRSRKWQRRFFILYEHGLLRYALDEMP TTLPQGTINMNQCTDVVDGEGRTGQKFSLC	120
Mouse: 61	NPVHRSRKWQRRFFILYEHGLLRYALDEMP TTLPQGTINMNQCTDVVDGE RTGQKFSLC	120
Human: 121	ILTPEKEHFIRAETKEIVSGWLEMLMVYPRTNKQNKQKKRKRKEPPTPQEPGPAKVAVTSS	180
Mouse: 121	ILTPDKEHFIRAETKEIISGWLEMLMVYPRTNKQNKQKKRKRKEPPTPQEPGPAKMAVTSS	180
Human: 181	S--SSSSSSSIPSAEKVPTTKSTLWQEEMRTKDQPDGSSLSPAQSPSQSPPAAASSLREP	238
Mouse: 181	S +S SSSSIPSAEKVPTTKSTLWQEEMR KDQPDG+SLSPAQSPSQSPPAA + REP	240
Human: 239	GLESKEEESAMSSDRMDCGRKVRVESGYFSLEKTKQDLKAEQQLPPLSPSPSTPNHR	298
Mouse: 241	GLESKE+ES +S DR+D GRKVRVESGYFSLEK QDL+AEEQ LPP LSPSPSTP+ R	299
Human: 299	RSQVIEKFEALDIEKAEHMETNA-VGPSPSSDTRQGRSEKRAFPRKRDFNEAPPAPLPD	357
Mouse: 300	RSQVIEKFEALDIEKAEHMETN + +PSSDTRQGRSE+RA PRKRDF +EAP APL D	359
Human: 358	ASASPLSPHRRAKSLDRRSTEPSVTDLLNFKKGWLTKQYEDGQWKKHWFVLADQSLRYY	417
Mouse: 360	AC--PLSPHRRAKSLDRRSTESSMTDLLNFKKGWLTKQYEDGQWKKHWFVLADQSLRYY	417
Human: 418	RDSVAEEAADLDGEIDL SACYDVTEYVPVQRNYGFQIHTKEGEFTLSAMTSGIRRNWIQTI	477
Mouse: 418	RDSVAEEAADLDGEINLSTCYDVTEYVPVQRNYGFQIHTKEGEFTLSAMTSGIRRNWIQTI	477
Human: 478	MKHVHPTTAPDVTSSLPEEKNKSSCSFETCPRPTEKQEAELGEPDPEQKTSRARERRREG	537
Mouse: 478	MKHV P +APDVTSSLPE KNKS+ SFETC R TEKQAE GEPDPEQK SRARERRREG	536
Human: 538	RSKTFDWAEFRPIQQALAQERVGGVGPADTHEPLRPEAESGELERERARRREERRKRFGM	597
Mouse: 537	RSKTFDWAEFRPIQQALAQER VG +D+ +P EAE GELERERARRREE RKRFGM	596
Human: 598	LDATDGPGTEDAALRMEVDRSPGL-PMSDLKTHNVHVEIEQRWHQVETTPLEEKQVPPIA	656
Mouse: 597	LD DGPG ED ALRM++DRSPGL DLKT NVHVEIEQRWHQVETTPLEEKQVPPIA	656
Human: 657	PVHLSSDGGDRLSTHELTSLLEKELEQSQKEASDLLEQNRLLDQLRVALGREQSAREG	716
Mouse: 657	P+HLS ED +RLSTHELTSLLEKELEQSQKEASDLLEQNRLLDQLRVALGREQSAREG	716
Human: 717	YVLQATCERGFAAMEETHQKKIEDLQRQHQRELEKLREEKDRLLAEETAATISAIEAMKN	776
Mouse: 717	YVLQATCERGFAAMEETHQKKIEDLQRQHQRELEKLREEKDRLLAEETAATISAIEAMKN	776
Human: 777	AHREEMERELEKSQRSQISSVNSDVEALRRQYLEELQSVQRELEVLSQYQSQKCLENAHL	836
Mouse: 777	AHREEMERELEKSQRSQISS+NSD+EALRRQYLEELQSVQRELEVLSQYQSQKCLENAHL	836

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Human: 837 AQALEAERQALRQCQRENQELNAHNQELNNRLAAEITRLRTLTLTGDDGGGEATGSPLAQGK 896  
AQALEAERQALRQCQRENQELNAHNQELNNRLAAEITRLRTLTLTGDDGGGE+TG PL QGK  
Mouse: 837 AQALEAERQALRQCQRENQELNAHNQELNNRLAAEITRLRTLTLTGDDGGGESTGLPLTQGK 896

Human: 897 DAYELEVLLRVKESEIQYLKQEISSLKDELQ TALRDKKYASDKYKDIYTELSIAKAKADC 956  
DAYELEVLLRVKESEIQYLKQEISSLKDELQ TALRDKKYASDKYKDIYTELSIAKAKADC  
Mouse: 897 DAYELEVLLRVKESEIQYLKQEISSLKDELQ TALRDKKYASDKYKDIYTELSIAKAKADC 956

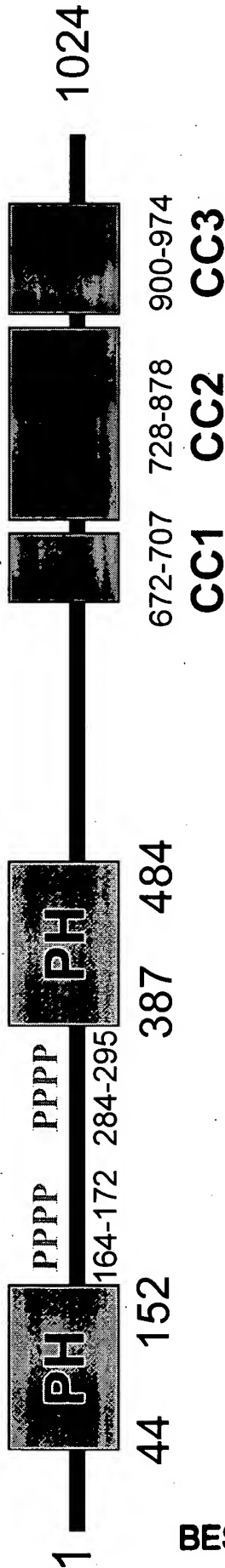
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DISRLKEQLKAATEALGEKSP+ TVSGYDIMKSKSNPDFLKKDRSCVTRQLRNIRSKSV  
Mouse: 957 DISRLKEQLKAATEALGEKSPEGTTVSGYDIMKSKSNPDFLKKDRSCVTRQLRNIRSKSV 1016

Human: 1017 IEQVSWDT 1024  
IEQVSWD  
Mouse: 1017 IEQVSWDN 1024

1B

# Yeast 2-Hybrid clones

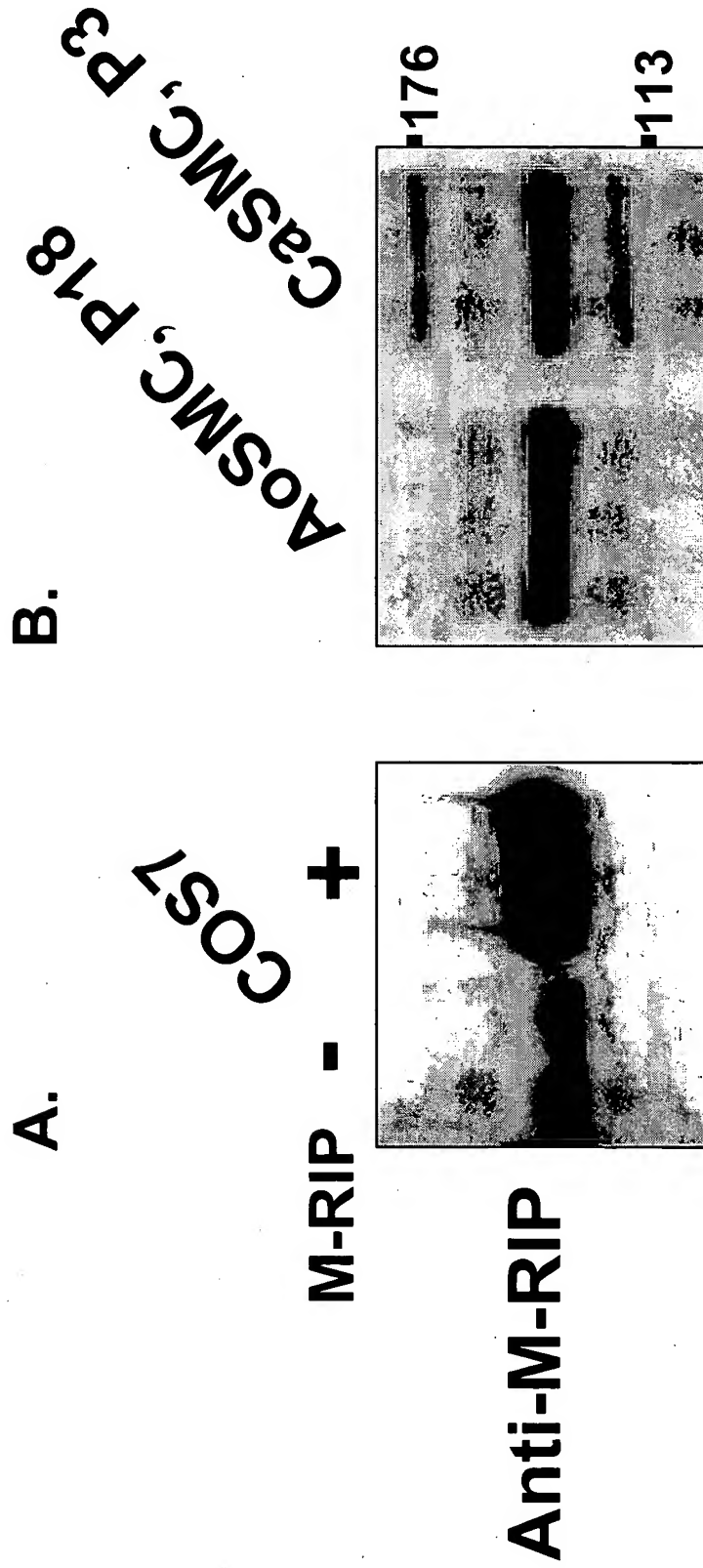
Clone 6 539 1024  
Clone 11 703 1024



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 ACCACAGGAGCCTGGGGCTTGCCCAAGGTGGCTGTTTACCAGCAGCAGCAGCAGCAGCAGCAGCAGCAGCATC  
 CCAGTGC TGAGAAAGTCCCCACCACCAAGTCCACACTCTGGCAGGAAGAAATGAGGACCAAGGACCAGCCCA  
 GATGGCAGCAGCCTGAGTCCAGCTCAGAGTCCAGCCAGAGCCAGCCTCCTGCTGCCAGCTCCCTGCGGGGA  
 ACCTGGGCTAGAGAGCAAGAAGAGGAGAGCGCCATGAGTAGCGACCCGATGGATGTTGGCCGCAAGTCC  
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 CAGAGCGCCCGTGAGGGCTACGTGCTGCAGGCCACGTGCGAGCGAGGGTTTGACAGCAATGGAAGAAACGCA  
 CCAGAAGAAGATTGAAGATCTCCAGAGGCAGCACACGCGGGAGCTAGAGAAACTTCGAGAAGAGAAAGACC  
 GCCTCCTAGCCGAGGAGACAGCGGCCACCATCTCAGCCATCGAAGCCATGAAGAACGCCCCACCGGGAGGAA  
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 GACTTGGGGGAGAAGTCCCCCTGACAGTGCCACGGTGTCCGGATATGATATAATGAAATCTAAAAGCAACCCCT  
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**Figure 2**



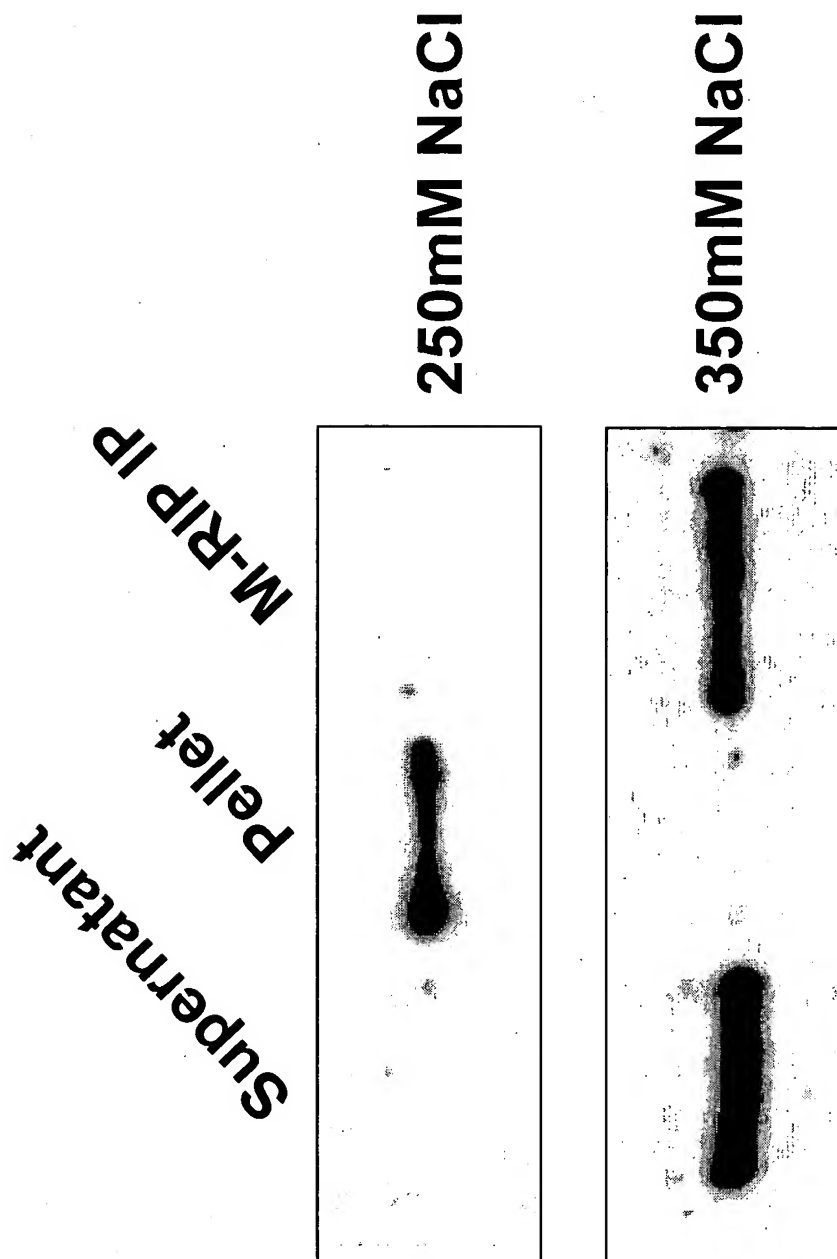
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2C

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Anti-M-RIP



2D

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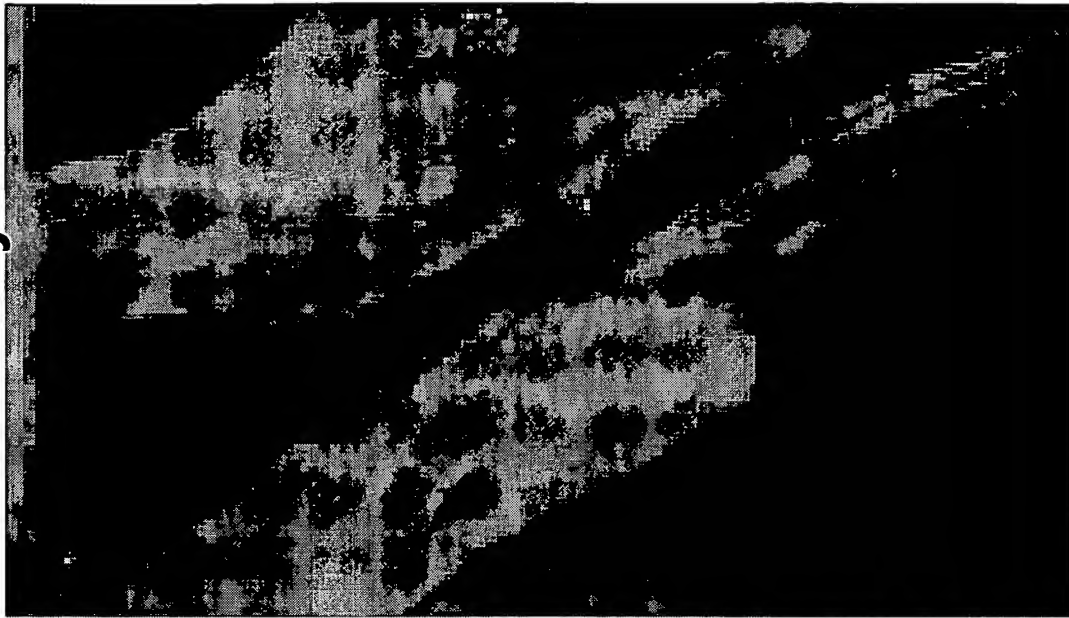
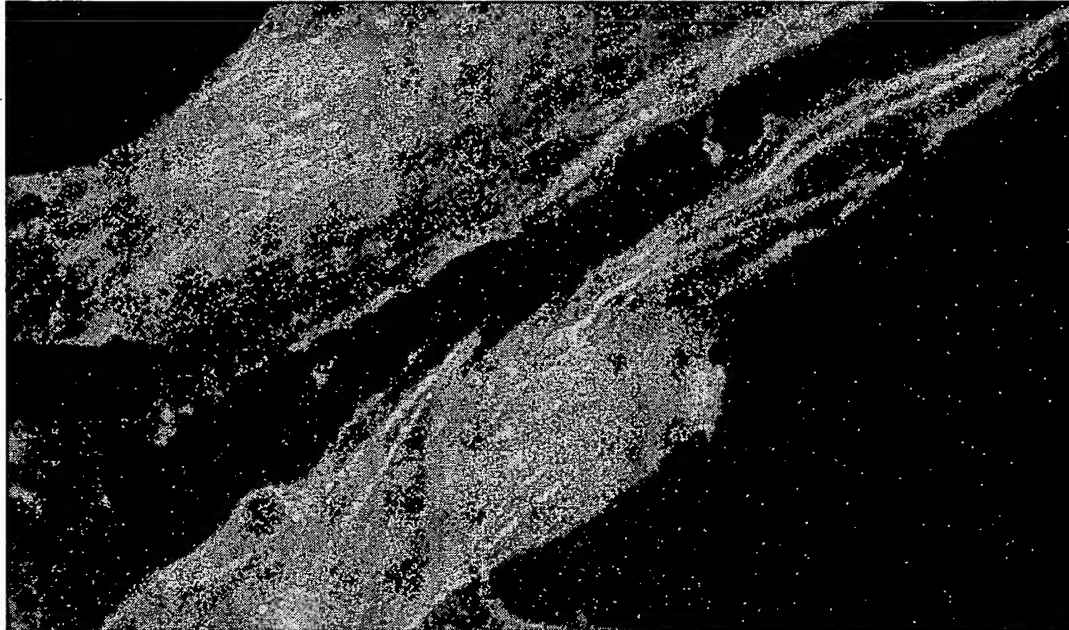
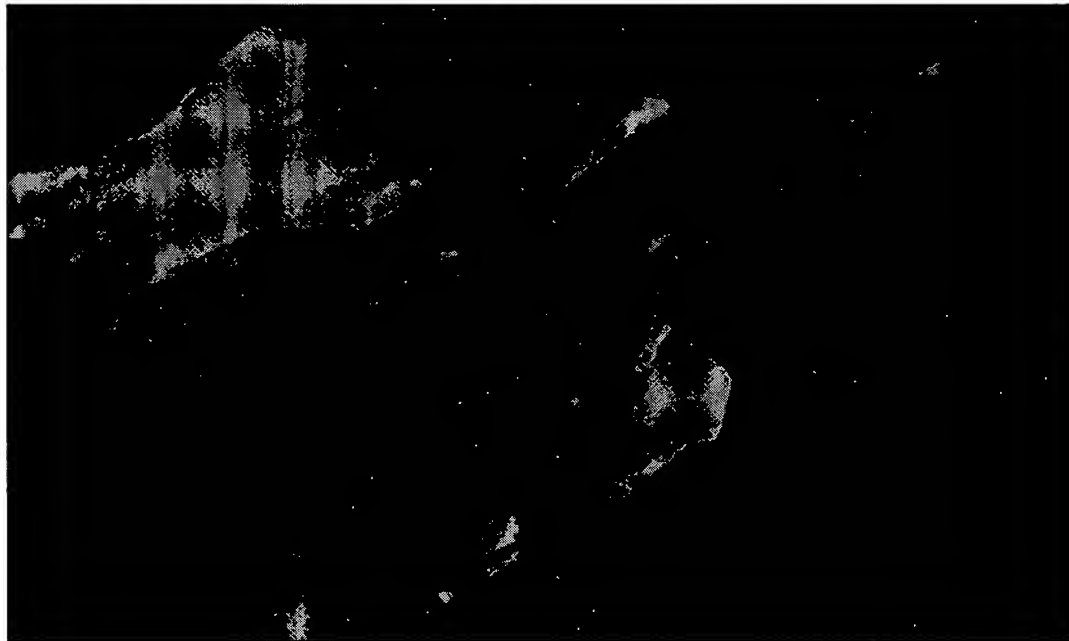
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Anti-M-RIP

Phalloidin

Overlay



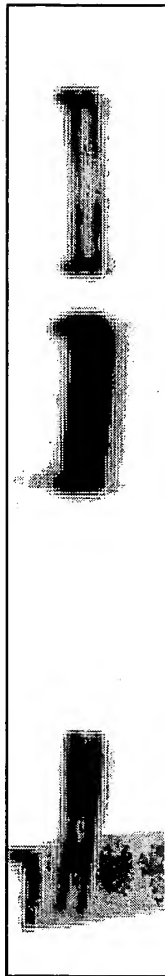
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3A

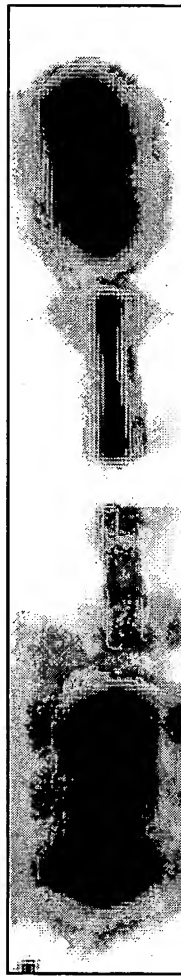
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5% Input  
Nonimmune IgG  
MBS IP  
M-RIP IP

Anti-MBS



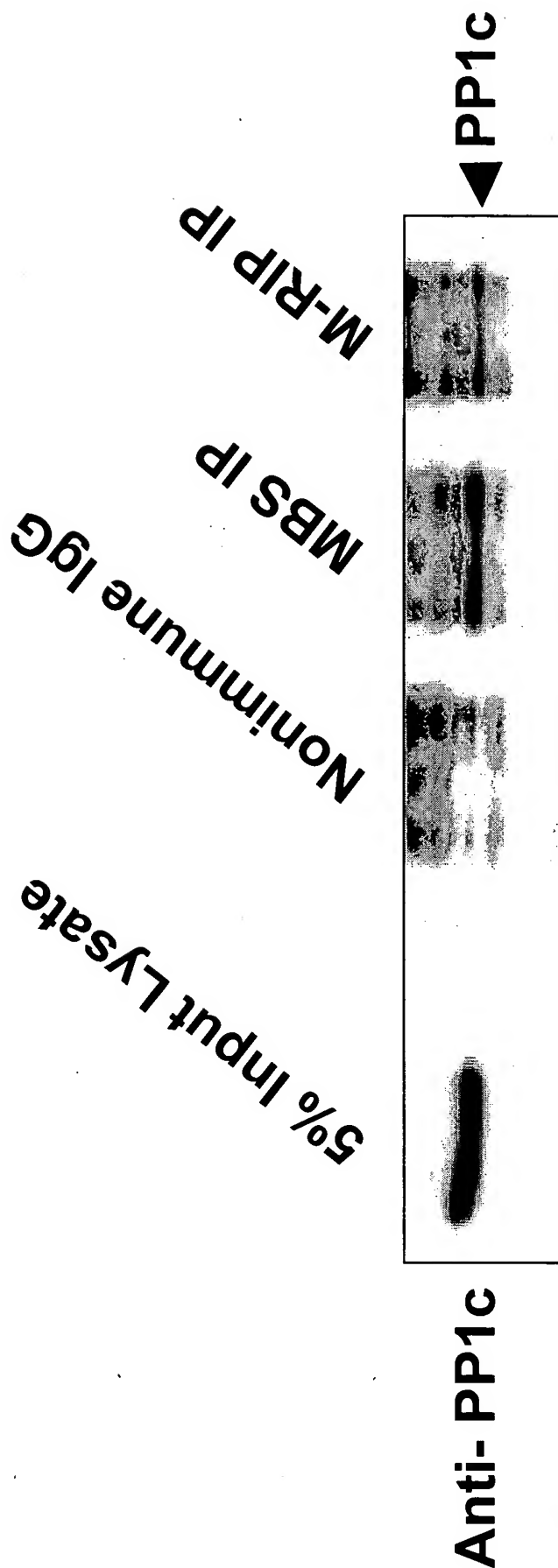
Anti-M-RIP





3B

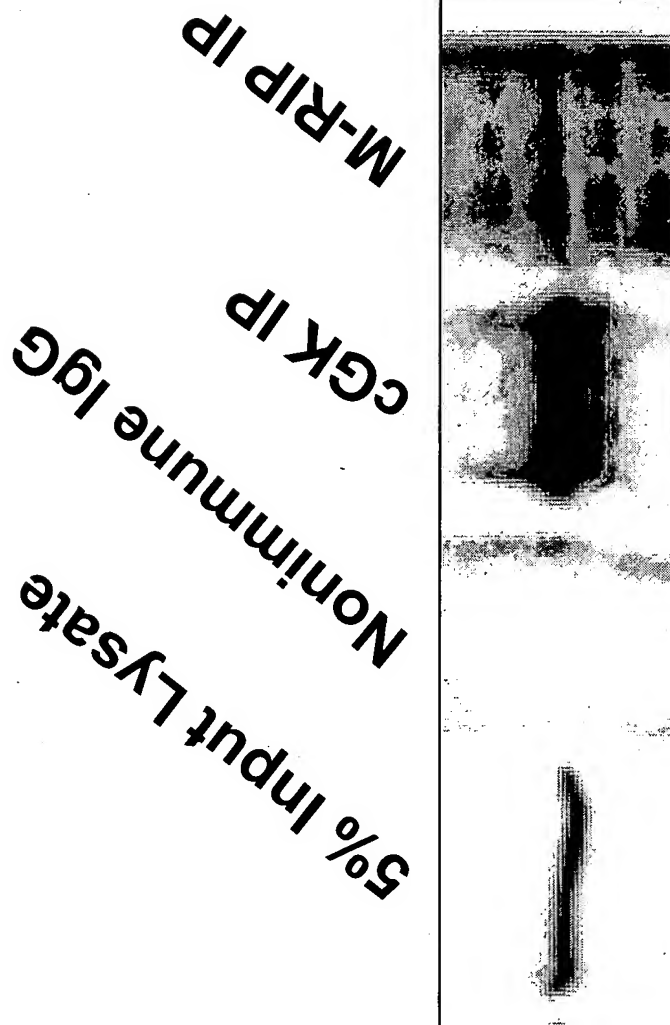
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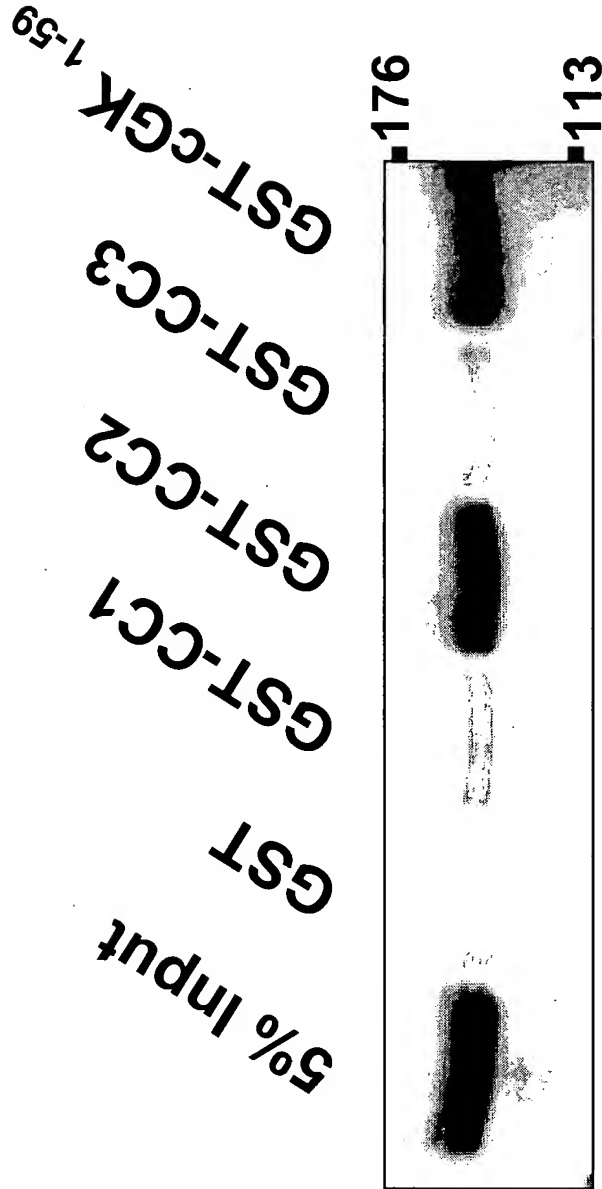
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# Anti- cGK



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# Anti-MBS

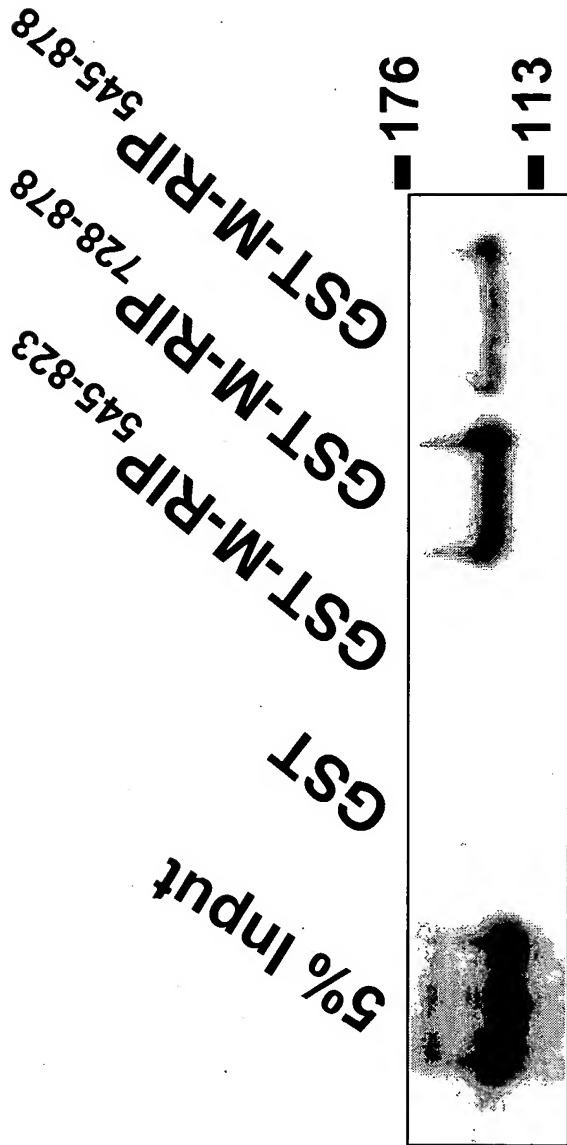


4B

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# Anti-MBS

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Amino Acids    MBS Binding

CC1    CC2    CC3



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672-707 (CC1)

-



728-878 (CC2)

+



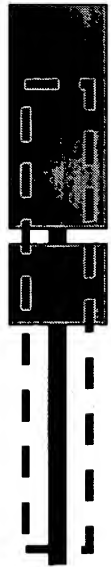
900-974 (CC3)

-



545-823

-

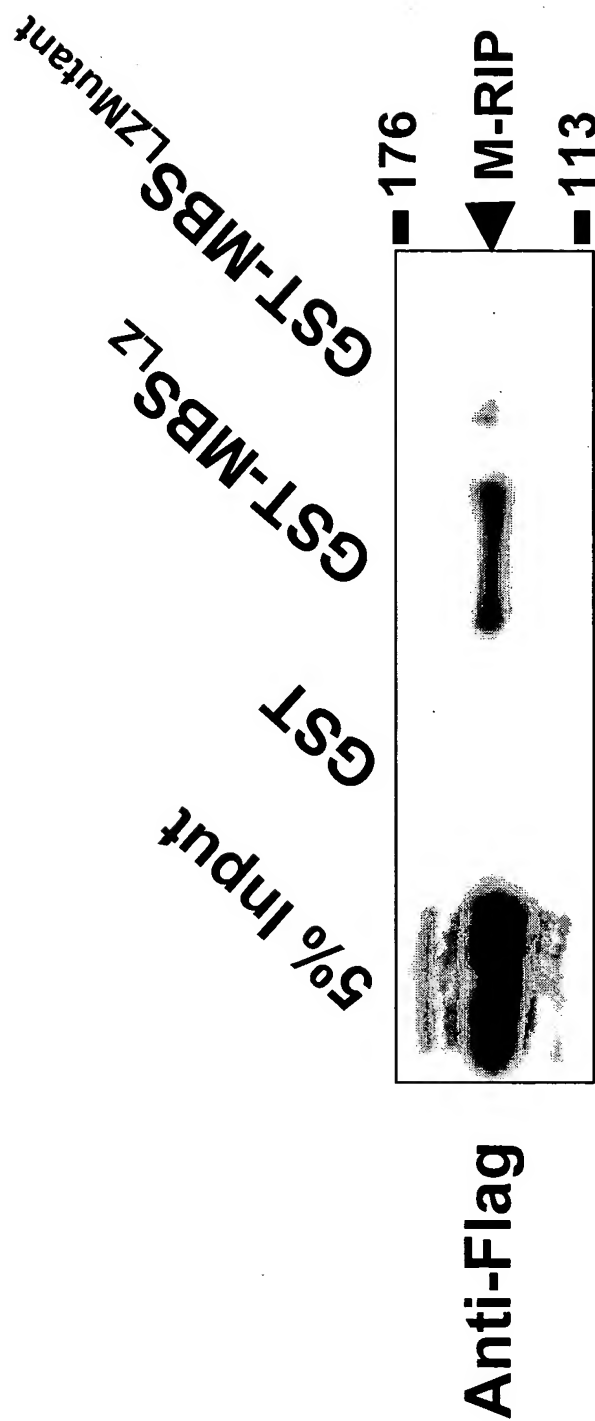


545-878

+

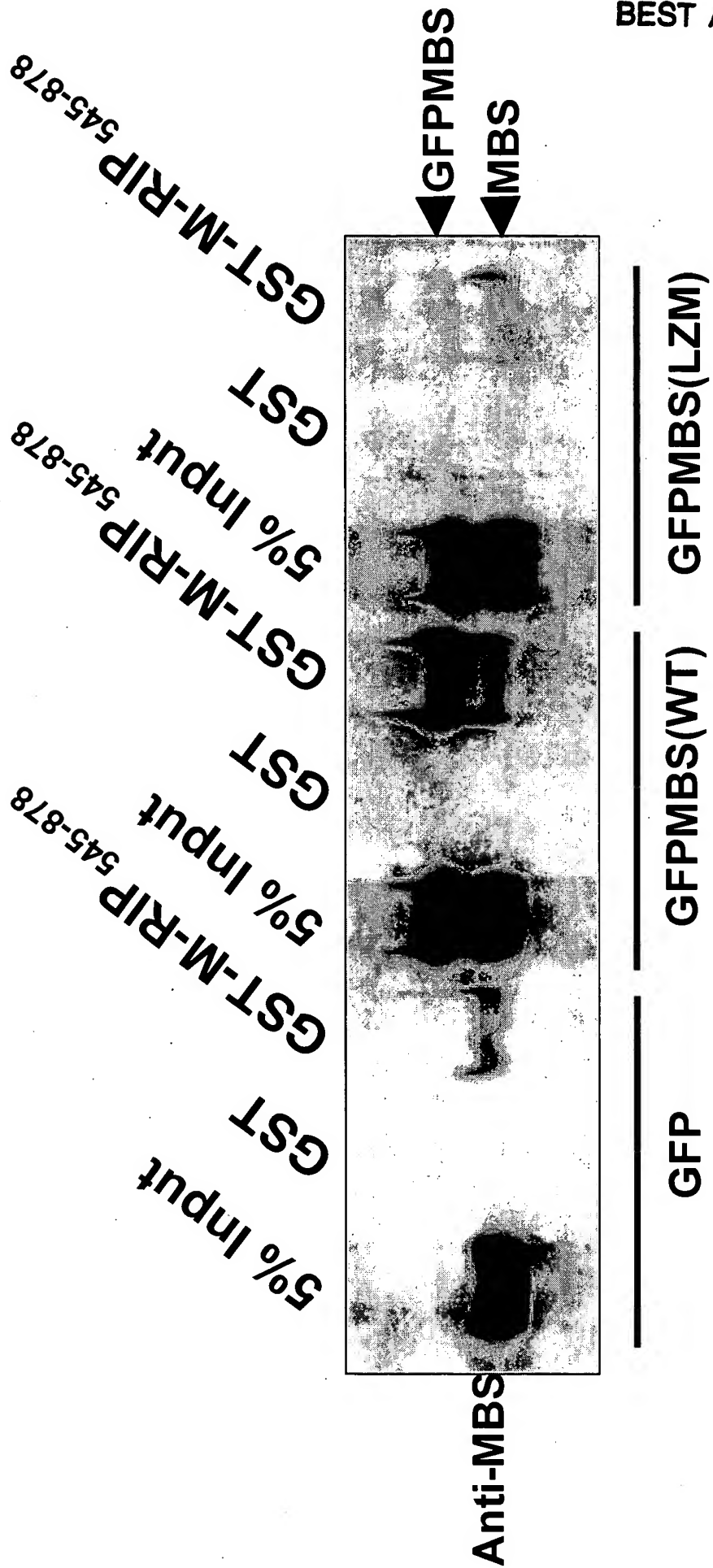
4D

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4E

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4F

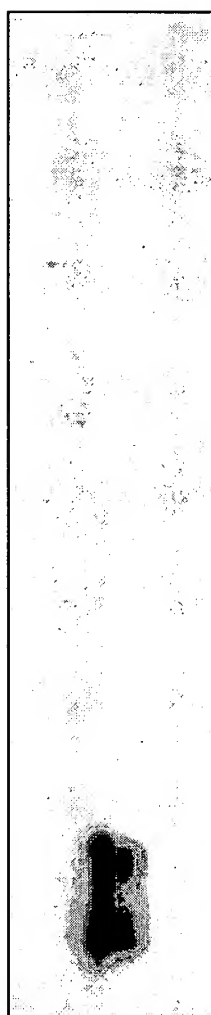
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Input  
Control  
HisCC1  
HisCC2  
HisCC3

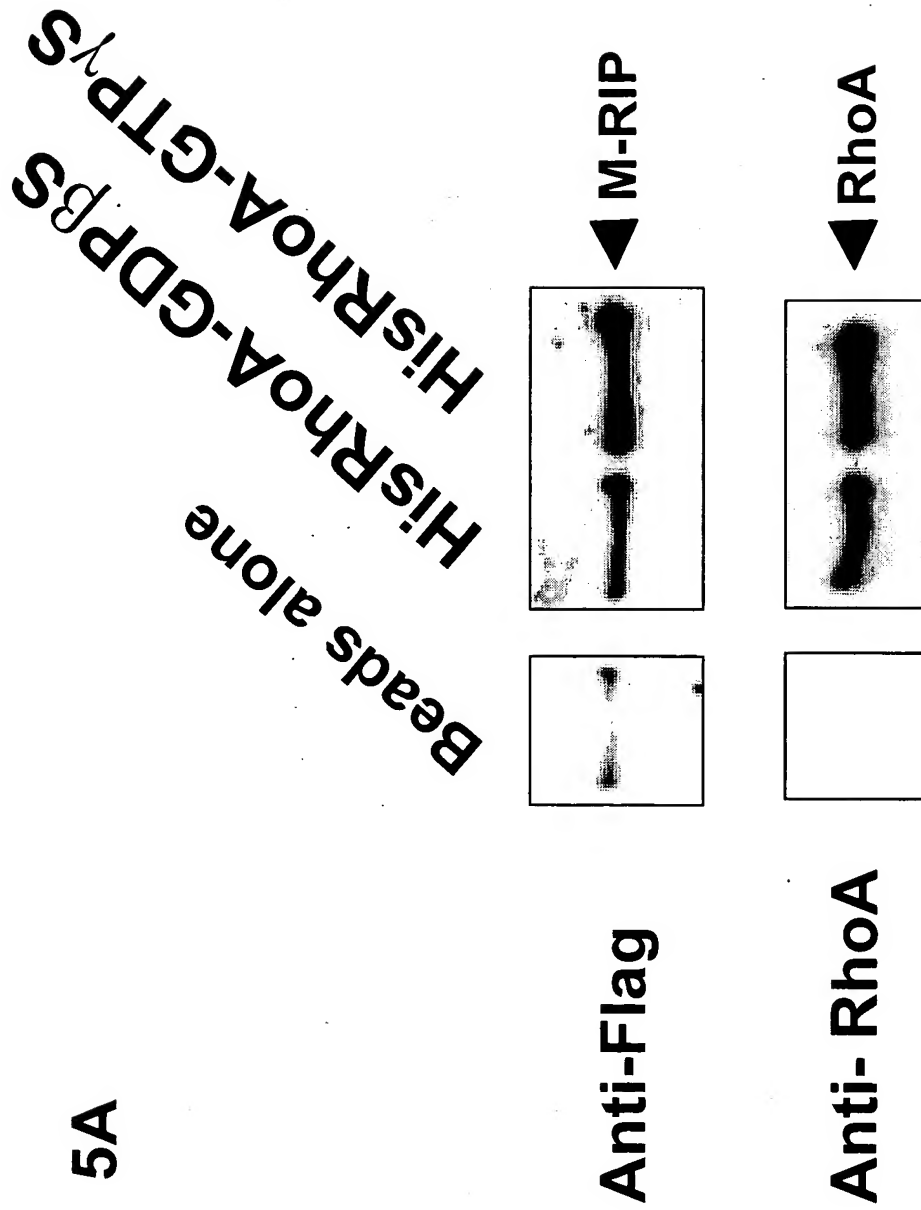
↓ GST-MBSLZ

↓ GST-MBSLZmutant



Anti-GST

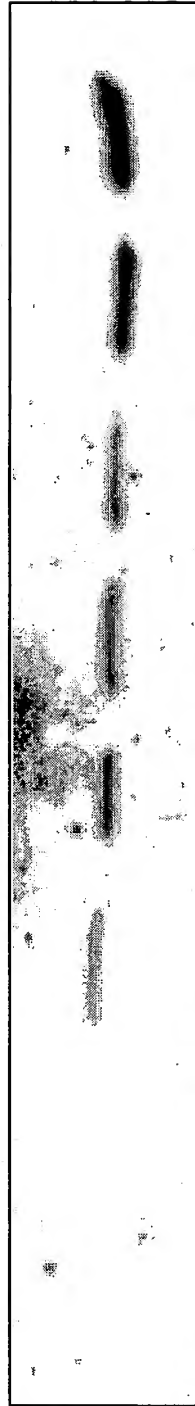




5B

GDP<sub>BS</sub> GTP<sub>YS</sub> GDP<sub>BS</sub> GTP<sub>YS</sub> GDP<sub>BS</sub> GTP<sub>YS</sub> GDP<sub>BS</sub> GTP<sub>YS</sub>

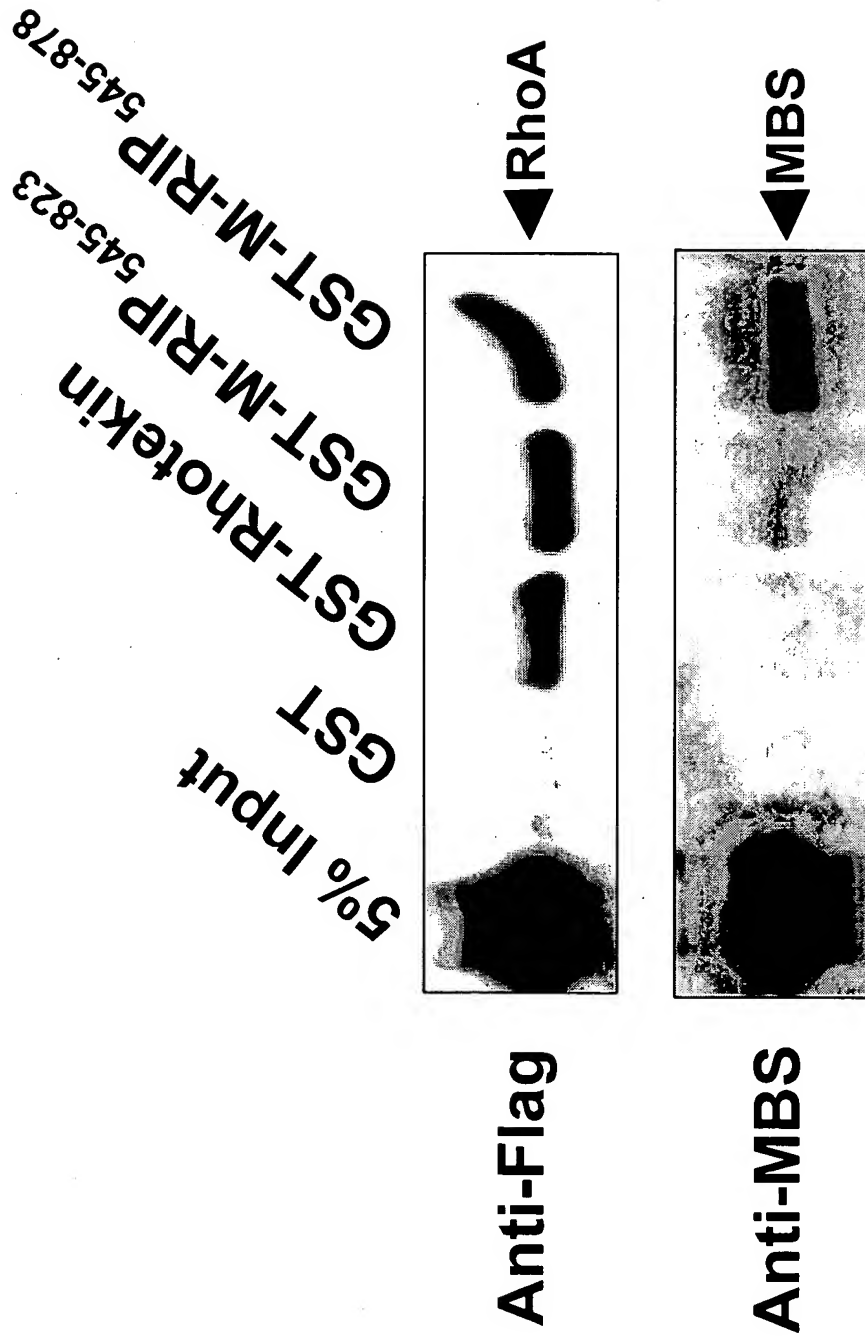
HisRhoA<sup>+</sup>:

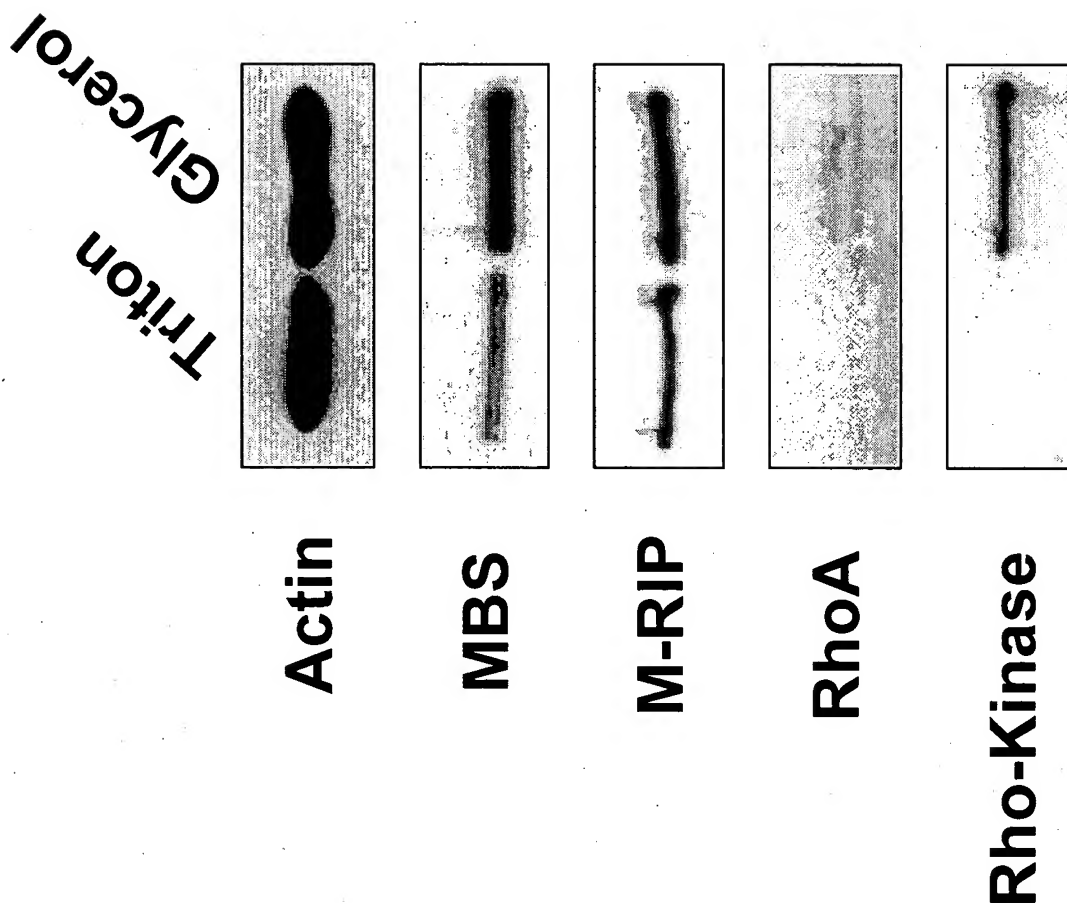


Anti-RhoA

_____	_____	_____	_____	_____
GST	GST-	GST-	GST-	GST-
	Rhotekin <sub>RBD</sub>	M-RIP <sub>545-823</sub>	M-RIP <sub>545-878</sub>	

5C





6A

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6B, top

Phalloidin



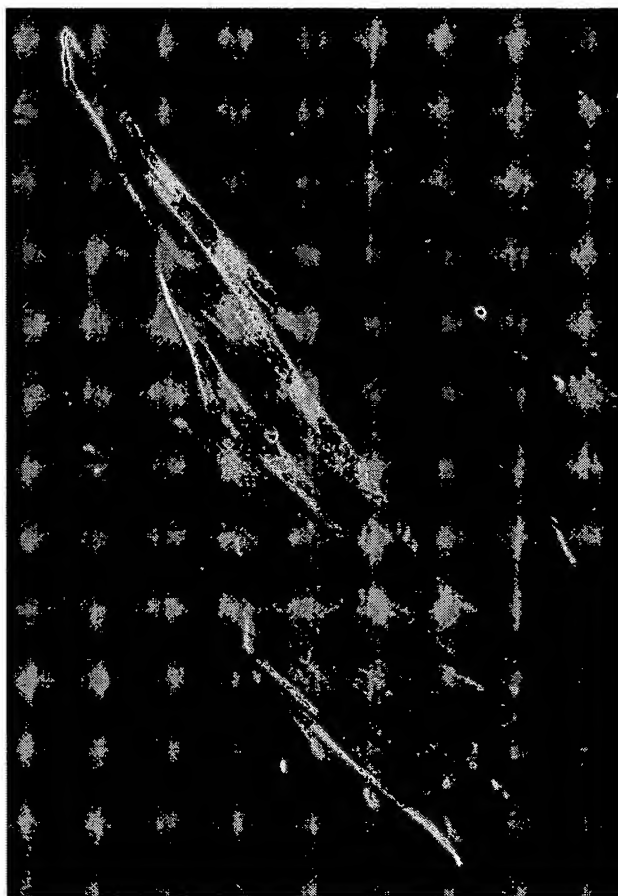
M-RIP



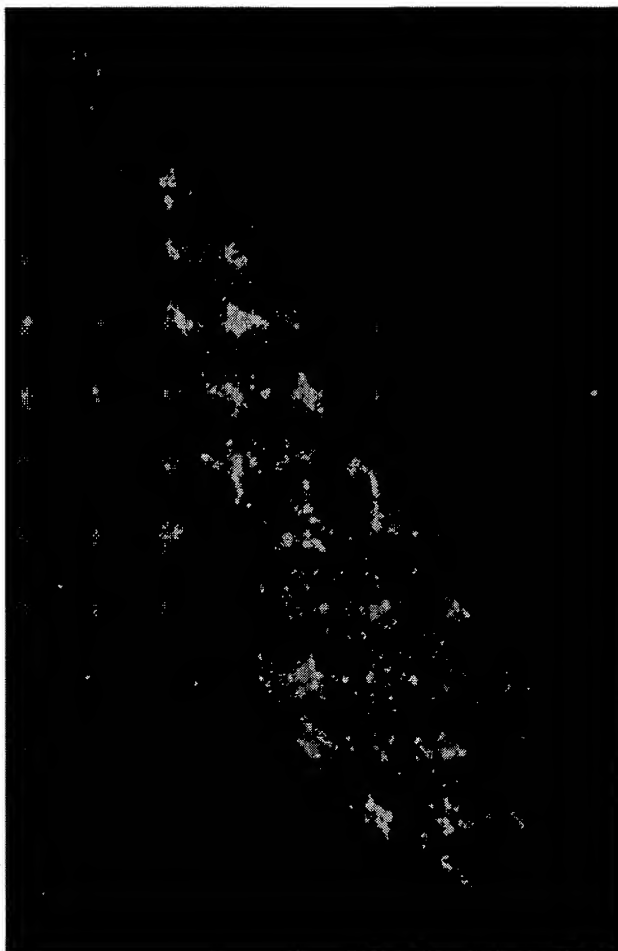
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**6B, middle**

**Phalloidin**



**MBS**



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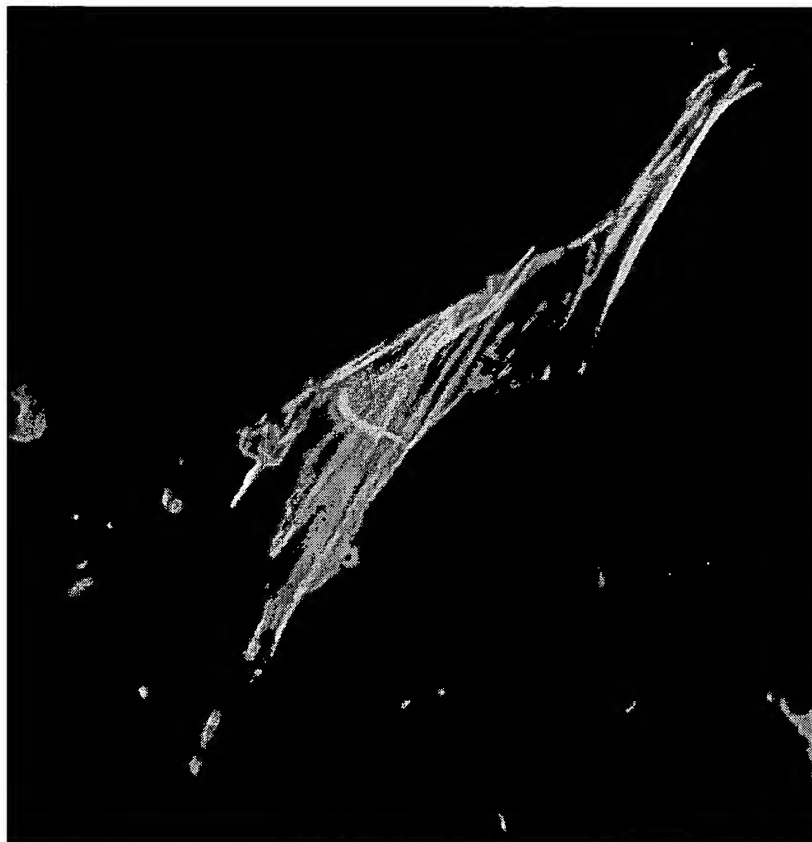
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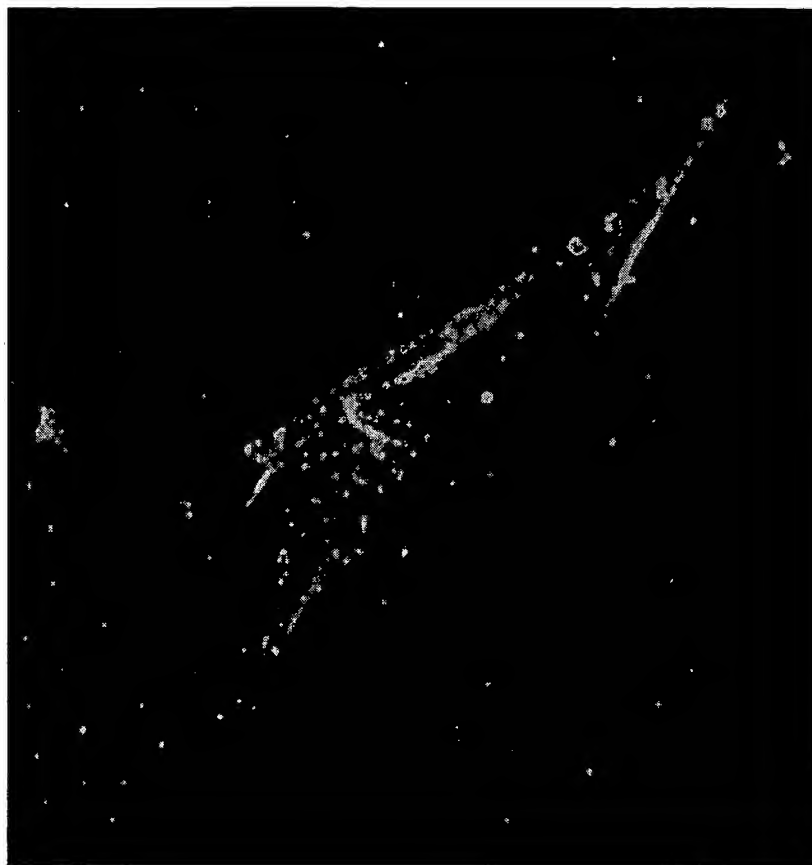
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6B, bottom

Phalloidin

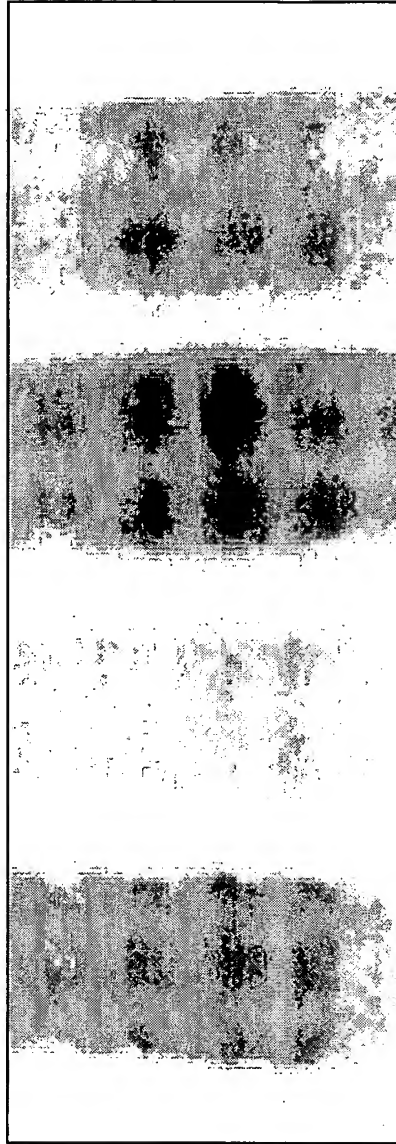


RhoA



6C

IP:NI IP:M-RIP



Y27632 1μM

+

-

+

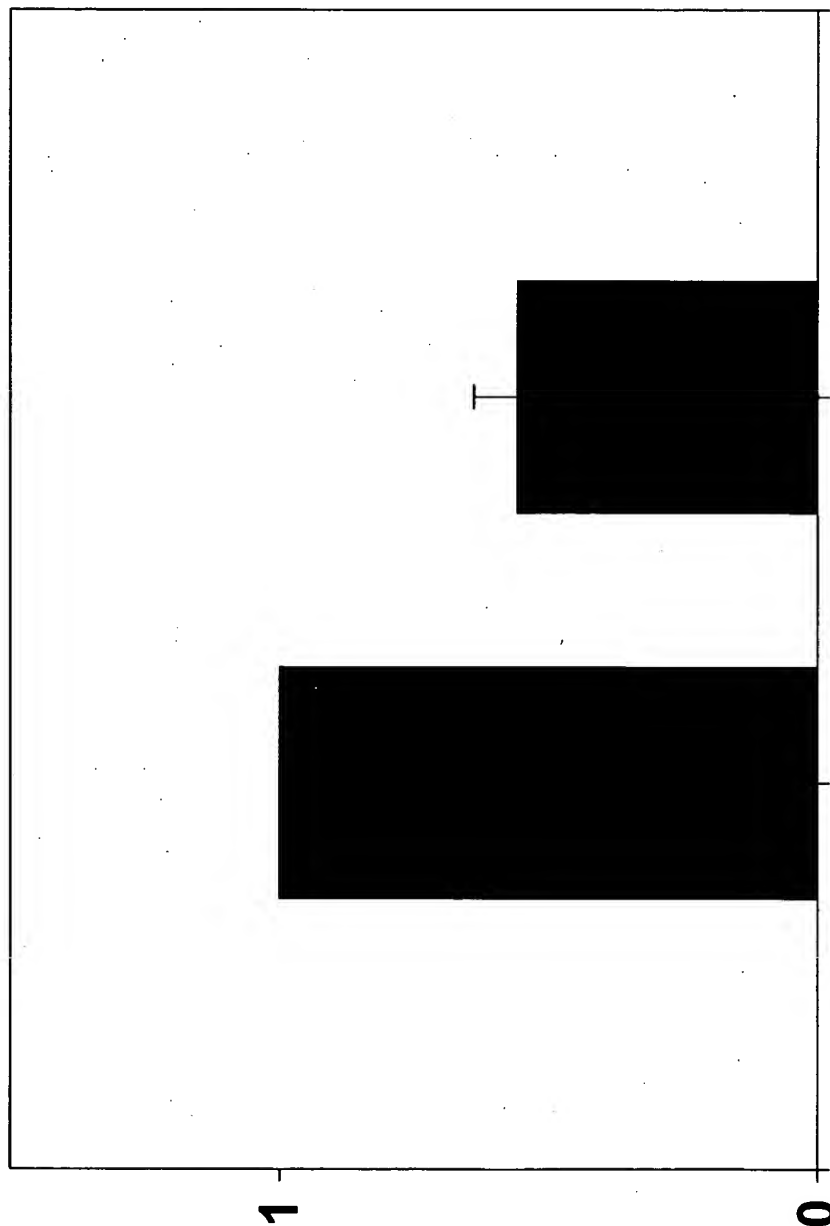
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6D

Arbitrary Densitometric Units



Y27632

